



Short communication

## A single dominant gene for downy mildew resistance in broccoli

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### Summary

Downy mildew, incited by *Peronospora parasitica* (Pers.: Fr.) Fr., is a destructive disease of broccoli (*Brassica oleracea* L., Italica Group). Resistant cultivars represent a desirable control method to provide a practical, environmentally benign, and long-term means of limiting damage from this disease. Doubled-haploid (DH) lines developed by us exhibit a high level of downy mildew resistance at the cotyledon stage. To determine the mode of inheritance for this resistance, a resistant DH line was crossed to a susceptible DH line to make an F<sub>1</sub>, from which F<sub>2</sub> and backcross (BC) populations were developed. All populations were evaluated for response to artificial inoculation with *P. parasitica* at the cotyledon stage. All F<sub>1</sub> plants (including reciprocals) were as resistant as the resistant parent, indicating no maternal effect for this trait. F<sub>2</sub> populations segregated approximately 3 resistant to 1 susceptible, BC populations using the resistant parent as the recurrent parent contained all resistant plants, and the BC to the susceptible parent segregated 1 resistant to 1 susceptible. These results indicate that resistance is controlled by a single dominant gene. This gene should be easily incorporated into F<sub>1</sub> hybrids and used commercially to prevent downy mildew at the cotyledon stage.

### Introduction

Downy mildew, incited by the fungus *Peronospora parasitica* (Pers.: Fr.) Fr., is an economically important disease of cruciferous crops (Channon, 1981). The disease has worldwide distribution having been reported on many important species in the family Brassicaceae and it can occur on all above-ground tissues (Sherf & MacNab, 1986; Channon, 1981). Broccoli (*B. oleracea*, Italica Group) is susceptible to infection at all growth stages; however, infection at the cotyledon stage can be particularly detrimental because seedling tissues are highly vulnerable to attack, and when infected, can result in stunted or killed plants. Fungicide applications can provide a means of controlling downy (Brophy & Laing, 1992), but registered fungicides may be lost in the future due to concerns about their possible negative environmental effects and concerns about food safety (Flint

et al., 1992). Resistant cultivars represent one possible alternative to fungicide use and could provide a practical, long-term solution to effective disease control.

Natti et al. (1967) studied two sources of downy mildew resistance in *B. oleracea* expressed at the cotyledon stage and found each to be controlled by a single dominant gene. Recently, Wang et al. (2001) characterized a high level of downy mildew resistance in a doubled haploid (DH) line of broccoli expressed at the 3–4 leaf stage that is controlled by two complementary, dominant genes. With a different resistance than described above, Hoser-Krause et al. (1987) identified a single recessive gene in broccoli responsible for downy mildew resistance at the 4–5-true leaf stage. Dickson & Petzoldt (1993) suggested that modifying genes probably act in concert with major genes to confer variable levels of downy mildew resistance. This is supported by observations of Jensen et al. (1999), who

characterized moderate resistance in breeding lines of broccoli.

Wang et al. (2000) showed that the hybrid broccoli cultivar Everest exhibits a high level of downy resistance at the cotyledon and true leaf stages. Parentage of 'Everest' is protected as a trade secret, and inheritance of its cotyledon stage resistance has never been described. Thus, we derived DH lines from 'Everest' and identified progeny lines with the same resistance but better suited for use in inheritance studies (Wang et al., 2000). The primary objective of this study was to determine inheritance of this resistance.

## Materials and methods

### Parental materials and genetic populations

Two DH broccoli lines (USVL089 and USVL047) developed by Farnham (1998) were evaluated in a previous study (Wang et al., 2000). USVL089 exhibits cotyledon-stage downy mildew resistance and is also resistant at later stages as well. This line was derived from the F<sub>1</sub> hybrid cultivar Everest (Syngenta Seed, Gilroy, CA) and served as the resistant (R) parent. USVL047 is susceptible to downy mildew at all stages, was derived from the F<sub>1</sub> hybrid cultivar Marathon (Sakata Seed Inc., Salinas, CA), and served as the susceptible (S) parent. The R and S parents were crossed in both directions to create two reciprocal F<sub>1</sub> hybrids (F<sub>1(RS)</sub> and F<sub>1(SR)</sub>), and these were used to: 1) generate four backcross (BC) populations (BC<sub>1(RS-R)</sub>, BC<sub>1(RS-S)</sub>, BC<sub>1(SR-R)</sub>, and BC<sub>1(SR-S)</sub>); and 2) make two F<sub>2</sub> populations [F<sub>2(RS)</sub> and F<sub>2(SR)</sub>].

### Downy mildew evaluation

A downy mildew resistance evaluation was performed at the cotyledon stage. This test consisted of 15 plants for each parent, 20 for each F<sub>1</sub>, 60 for each BC<sub>1</sub>, and 100 for each F<sub>2</sub>. Growth of seedlings, inoculation of cotyledons, incubation of inoculated seedlings, and rating of seedlings for downy mildew reaction phenotype (RP) were all conducted as described in our previous study (Wang et al., 2000). A single South Carolina isolate of *P. parasitica* isolated from field-grown plants of broccoli (Thomas & Jourdain, 1990) was used as inoculum. Using our RP rating (Wang et al., 2000), seedlings with a score of less than 3 (on a 0–9 scale), lacking any sporulation, were designated as resistant, and seedlings with 3 or higher, exhibiting sporulation, were considered susceptible. Data were

Table 1. Segregation of downy mildew resistant and susceptible plants in reciprocal F<sub>1</sub>, F<sub>2</sub> and BC<sub>1</sub> populations derived from the cross of USVL089 (R) and USVL047 (S) parents. Expected ratios for resistant versus susceptible are based on the model with a single dominant gene conditioning resistance

Genotype	Observed plants		Expected ratio	$\chi^2$	P
	R	S			
USVL089 (R)	15	0			
USVL047 (S)	0	15			
F <sub>1(RS)</sub>	20	0			
F <sub>1(SR)</sub>	20	0			
F <sub>2(RS)</sub>	77	23	3:1 (75:25)	0.21	0.5–0.8
BC <sub>1(RS)R</sub>	60	0	1:0 (60:0)		
BC <sub>1(SR)S</sub>	30	30	1:0 (30:30)		
F <sub>2(SR)</sub>	76	24	3:1 (75:25)	0.05	0.8–0.9
BC <sub>1(SR)R</sub>	60	0	1:0 (60:0)		
BC <sub>1(SR)S</sub>	27	33	1:1 (30:30)	1.65	0.5–0.3

collected on individual seedlings. Chi-square tests were used to determine goodness-of-fit to hypothesized models based on observed to expected numbers of resistant and susceptible individuals in the F<sub>2</sub> and BC.

## Results and discussion

All plants of the USVL047 parent were uniformly and highly susceptible to infection by *P. parasitica* at the cotyledon stage (mean RP = 8.9). Conversely, all plants of the USVL089 parent were uniformly and highly resistant (mean RP = 0.2). All F<sub>1</sub> plants from reciprocal crosses were highly resistant, and there was no significant difference in RP rating between F<sub>1(RS)</sub> and F<sub>1(SR)</sub> (mean RP = 0.9 for both). The high level of resistance expressed by reciprocal F<sub>1</sub>s indicates dominant gene action.

The F<sub>2(RS)</sub> population segregated 77 resistant to 23 susceptible, and the reciprocal F<sub>2(SR)</sub> population gave similar results, segregating 76 resistant to 24 susceptible (Table 1). The reciprocal BC populations to the susceptible parent were similar to each other, with BC<sub>1(RS-S)</sub> segregating 30 resistant to 30 susceptible and BC<sub>1(SR-S)</sub> segregating 27 resistant to 33 susceptible. Both reciprocal BC populations to the resistant parent did not segregate and exhibited only resistant plants. Chi-square tests revealed a close fit to a 3:1 ratio of resistant to susceptible for F<sub>2</sub> populations. BC populations using USVL047 (S) as recurrent parent fit

a 1 resistant to 1 susceptible ratio. The above data indicate a single dominant gene in USVL089 confers the cotyledon stage resistance. In addition, because reciprocal  $F_1$ ,  $F_2$ , and BC populations exhibited the same response or segregation ratios, no maternal effects are indicated for the single dominant gene.

Natti et al. (1967) cited two different single dominant genes identified from different *B. oleracea* sources (one cabbage and one broccoli U.S. plant introduction) and concluded that the two genes were effective against different races of *P. parasitica* at the cotyledon stage. We are unaware of any evidence indicating the genes described by Natti et al. (1967) were ever utilized in commercial *B. oleracea* crops or that the resistant plants identified were ever perpetuated and released. Thus, it is currently impossible to determine if one of the genes described by Natti et al. (1967) might be the gene in USVL089. This appears unlikely however, because one breeder (J. Stern, personal communication) who participated in the development of 'Everest' has indicated that the parentage of this hybrid included no germplasm originating from Natti or his colleagues.

Because cotyledon stage resistance in USVL089 is conditioned by a single gene, this resistance will prove easy to transfer to new breeding lines. Although it has not been stressed in this discussion, it is important to note that USVL089 is also resistant beyond the cotyledon stage (Wang et al., 2000). Thus, resistance in this line protects against damage by downy mildew as true leaves develop. The dominant expression of resistance in USVL089 will allow it, and other lines that contain the same resistance (Wang et al., 2000), to be used as parent inbreds for developing resistant  $F_1$  hybrids. The dominant resistance genes of USVL089 are especially useful because they need to be incorporated into only one of the parental inbreds to be effective. Also, a lack of maternal control for this dominant resistance allows resistant parents to be used as either a male or female in hybrid combinations. Thus, the cotyledon stage resistance described herein could be deployed readily in new commercial broccoli cultivars.

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